Table S1, Related to Figure 1:

Individual	**40291IMID**	**LIS6801**	**PAC3301**	**LIS7901**	**LIS8800**	**PAC2801**	**DC7401**
Sex at birth	Male	Female	Male	Female	Male	Female	Male
MRI abnormalities	BPPP,CSpZ, DCC, DWM	BPPP, CSpZ, DCC, DWM	BPPP,CSpZ, DCC, DWM, Chiari I	BPPP,CSpZ, DCC, DWM	BPPP,CSpZ, DCC, DWM	BPPP, CSpZ, DCC, DWM	BPPP,CSpZ, VL, DCC, Chiari I
Seizures	ES	ES	ES	Focal	Focal	Focal, GTC	Focal
Neurodevelopmental diagnoses	GDD	GDD	GDD, Echolalia	GDD, ID	GDD, ADHD, ASD, Anxiety	GDD, ADHD, ASD, SIB, ID	ID
Visual/Ocular	None	Unknown	Esotropia, Amblyopia, Hypermetropia	Esotropia, Hypermetropia	Convergence insufficiency	Exotropia	Exotropia
Neurological Exam	Hypertonia	Hypertonia	Normal tone	Hypotonia, Dysmetria	Hypotonia	Spasticity, Hypertonia	Broad gait
Head Circumference	50.5 cm (53%ile)	50.25cm (47%ile)	49.5cm (64%ile)	52.5cm (75%ile)	54 cm (88%ile)	51.5cm (41%ile)	Unknown
Evaluation Age	3y11m	5y1m	2y3m	8y	8y9m	8y11m	21yo

Table of phenotypes exhibited by individuals with *CEP85L* variants. BPPL: bilateral posterior predominant pachygyria, CSpZ: cell sparse zone, DCC: dysmorphic corpus callosum, DWM: decreased white matter, VL: volume loss, GTC: generalized tonic clonic, ES: epileptic spasms, GDD: global developmental delay, ADHD: attention deficient hyperactive disorder, ASD: autism spectrum disorders, SIB: self-Injurious behavior, ID: intellectual disability

Table S2, Related to Figure 1:

Individual	PAC2801	LIS7901/LIS8801	LIS6801	40291IMID	DC7401	PAC3301
Genomic Coordinates (GRch37/hg19)	chr6:g.118972428C>T	chr6:g.118953611C>A	chr6:g.118953675C>T	chr6:g.118953666G>A	chr6:g.118953654T>G	chr6:g.118953649C>A
cDNA position (Transcript NM_001042475.3)	c.5G>A	c.232+5G>T	c.173G>A	c.182C>T	c.194A>C	c.199G>T
Protein Position	p.Trp2*	N/A	p.Ser58Asn	p.Ser61Phe	p.Asp65Ala	p.Gly67Cys
Location	Exon 1	Intron 2	Exon 2	Exon 2	Exon 2	Exon 2
SIFT (Prediction Score)	N/A (nonsense variant)	N/A (intronic variant)	Deleterious (score: 0)	Deleterious (score: 0)	Deleterious (score: 0)	Deleterious (score: 0)
MutationTaster (Prediction Score)	Disease causing (prob: 1)	N/A (intronic variant)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)
PolyPhen-2 (HumVar)	N/A (nonsense variant)	N/A (intronic variant)	PROBABLY DAMAGING (0.997)	PROBABLY DAMAGING (0.998)	PROBABLY DAMAGING (0.998)	PROBABLY DAMAGING (1.000)
CADD (GRch37-v1.4)	17.24	20.7	26.2	28	27.1	28.8
M-CAP (Prediction score)	N/A (nonsense variant)	N/A (intronic variant)	Likely Benign (0.015)	Possibly Pathogenic (0.035)	Possibly Pathogenic (0.049)	Likely Benign (0.020)
PhastCons (44-vertebrates)	1	0.99	1	1	1	1
PhyloP (44-vertebrates)	1.5	4.4	4.4	4.4	3.68	4.4
gnomAD	0	0	0	0	0	0
1000Genomes	0	0	0	0	0	Ta0

Figure S1: Related to Figure 3

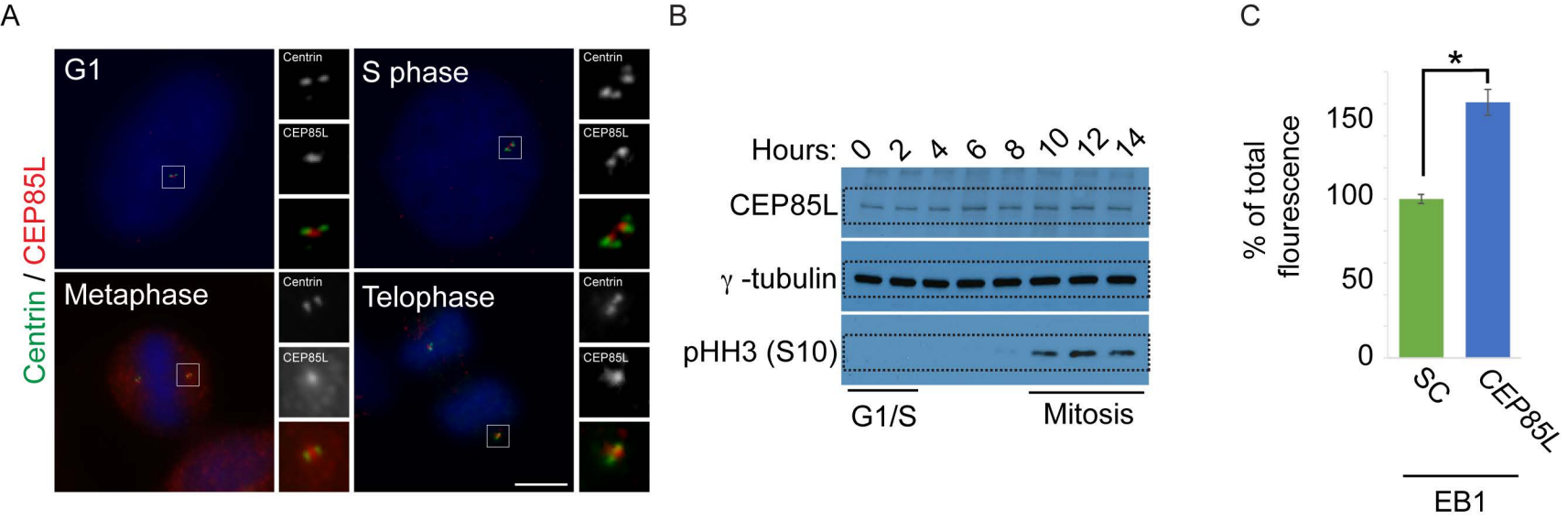
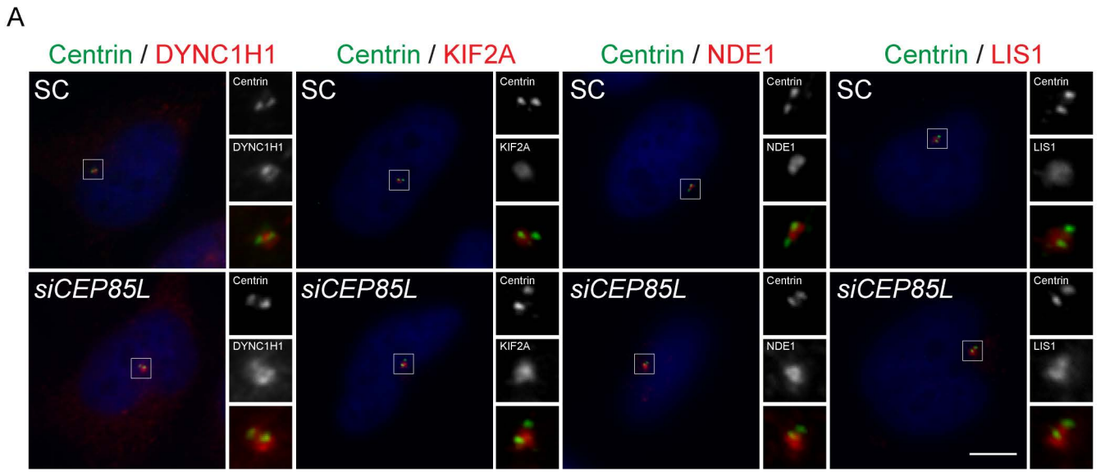
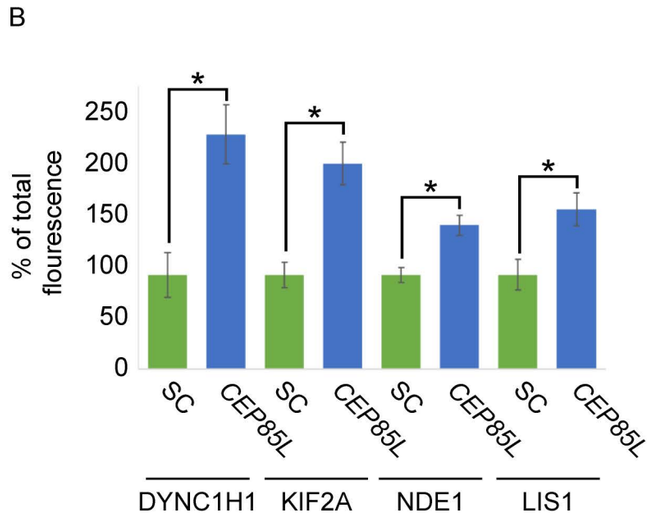


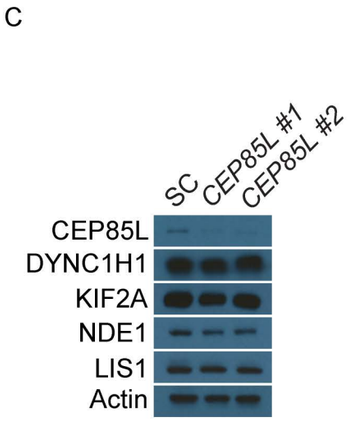
Figure S1: CEP85L is a centrosomal protein, Related to Figure 3

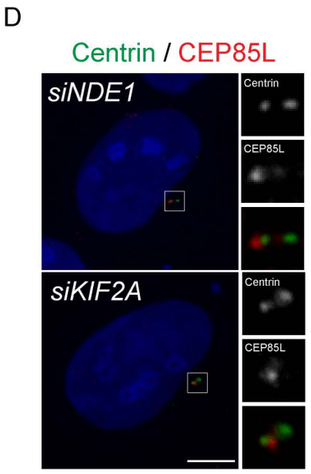
A. Asynchronous U2-OS cells co-stained for CEP85L (red) and Centrin (green) to determine the stage of the cell cycle. Scale bars represent 5μm for all images. **B.** Whole-cell lysates from thymidine-synchronized U2-OS cells harvested at the indicated time points and analyzed by immunoblot for CEP85L and phospho-Histone H3 (Ser 10). γ-tubulin served as a centrosomal loading control. **C.** Quantification of the fluorescence intensity of EB1 within 5μM of the centrosome expressed as the mean percentage ± s.d. of the fluorescence intensities of SC cells. For all quantifications, 10 cells were analyzed per experiment (n=3). * - $p < 0.005$ (Student's t-test).

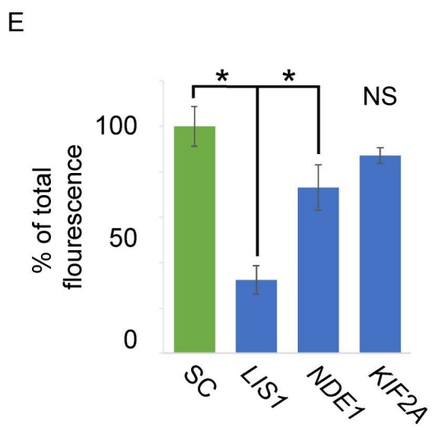
Figure S2: Related to Figure 4

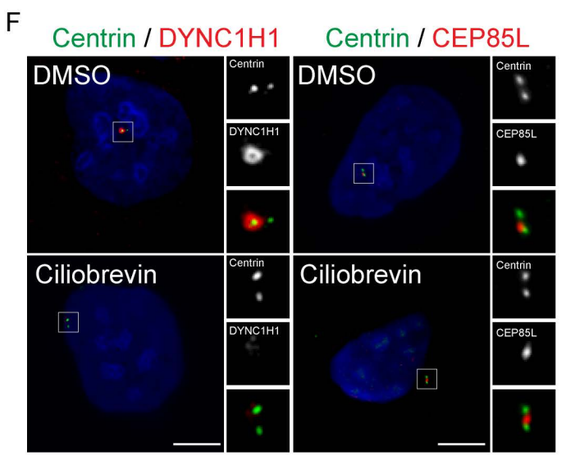


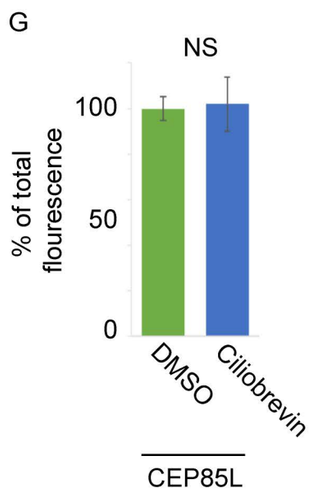


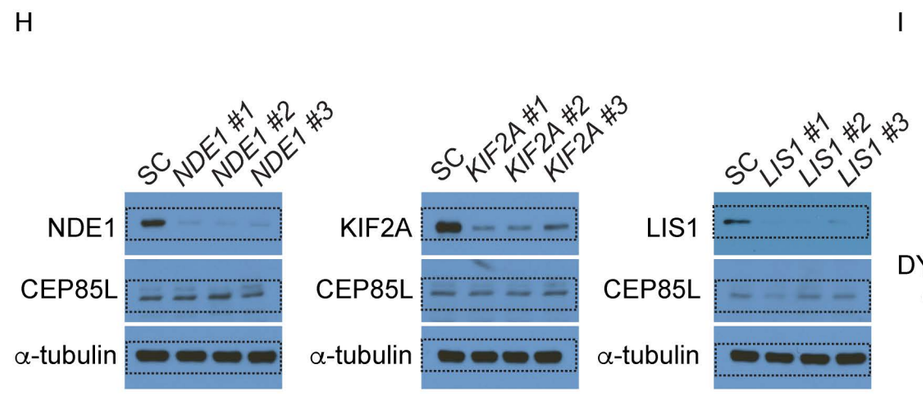


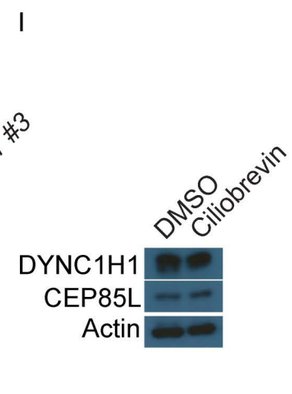


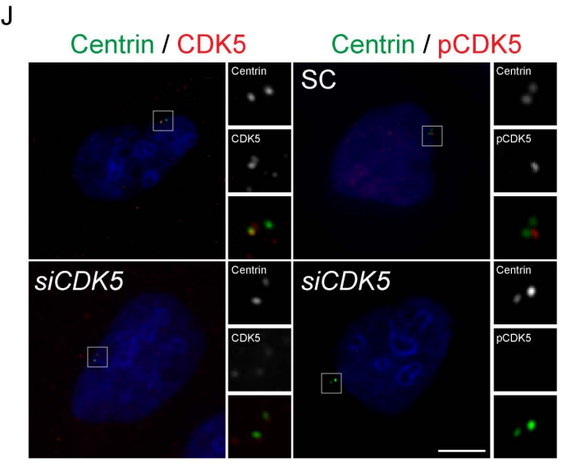


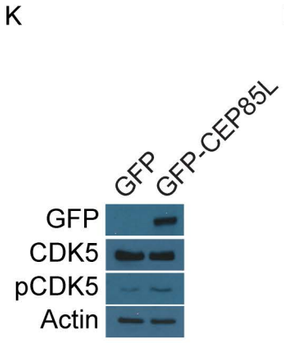


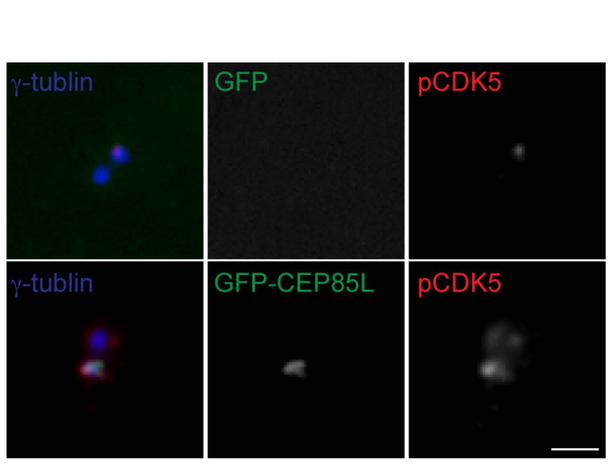


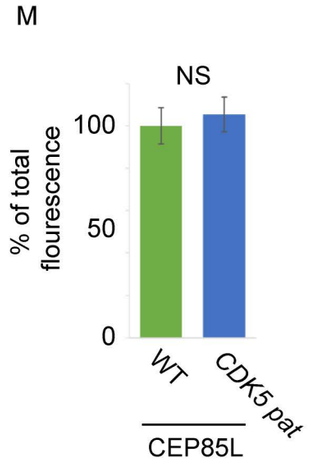


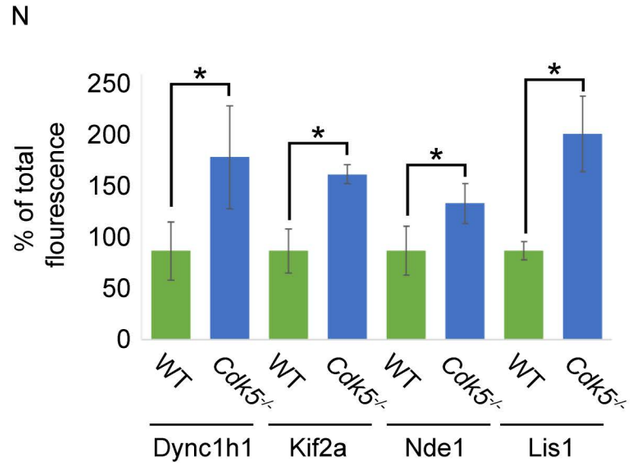


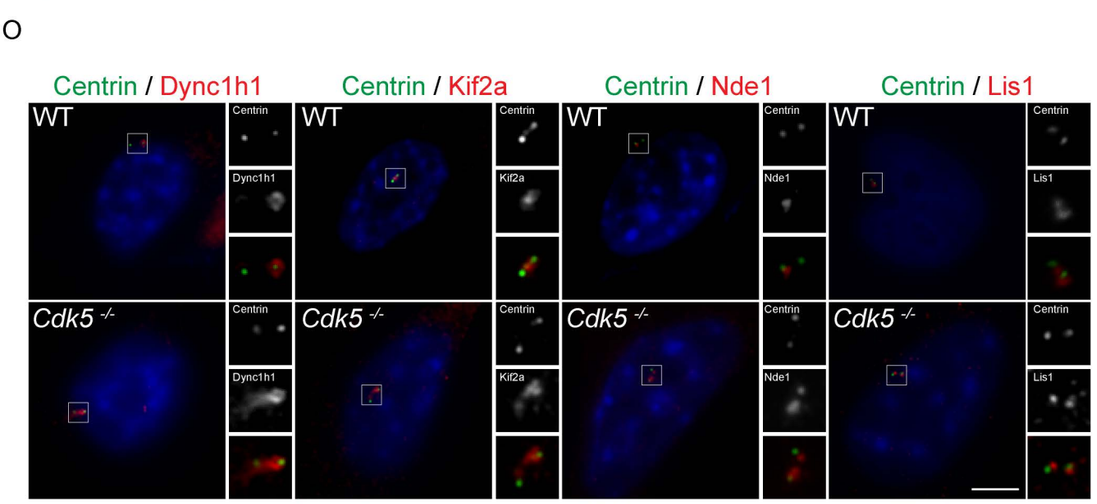


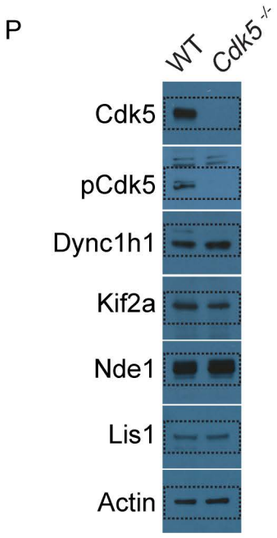


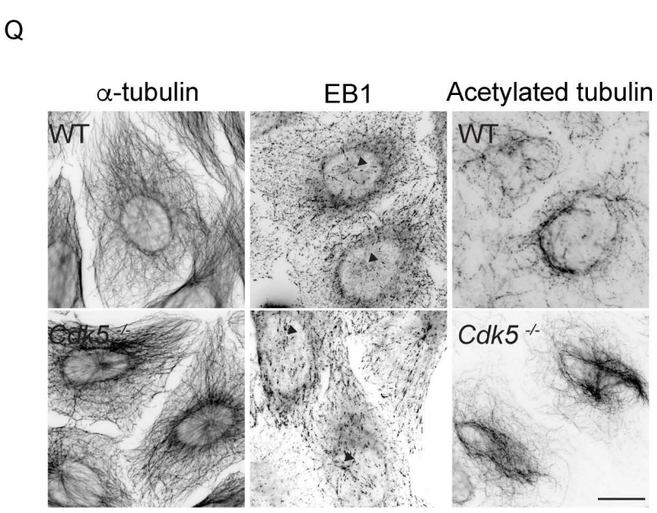


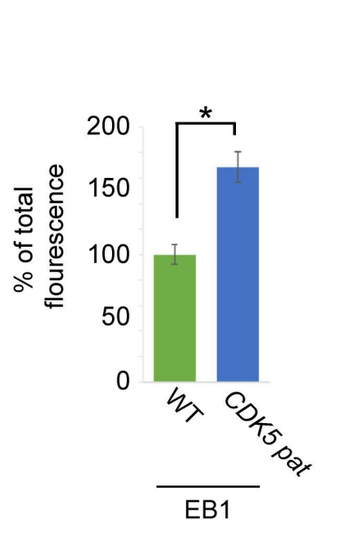


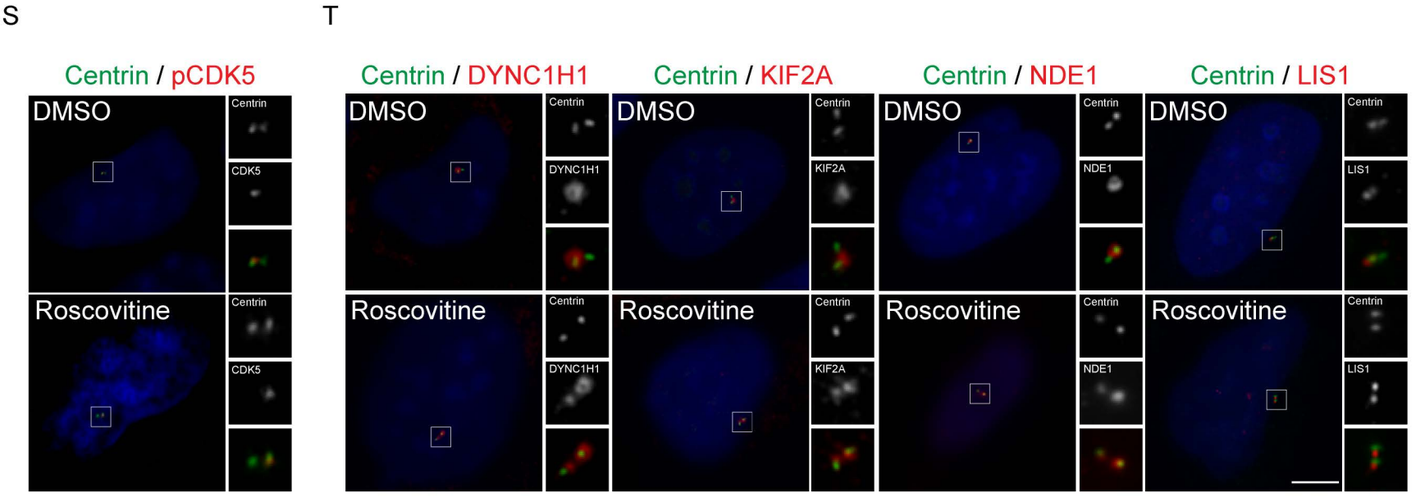


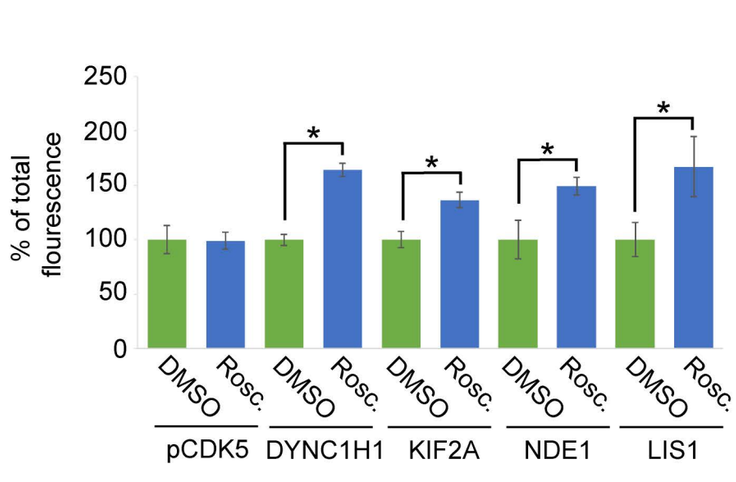












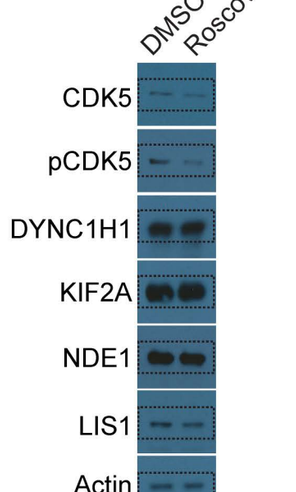


Figure S2: CEP85L is required to localize and activate CDK5 at the centrosome, Related to Figure 4

A. U2-OS cells transfected with SC or *CEP85L #2* siRNA co-stained for Centrin (green) and DYNC1H1, KIF2A, NDE1 or LIS1. Scale bars represent 5μm for all images. **B.** Quantification of the mean fluorescence intensities ± s.d. of centrosomal DYNC1H1, KIF2A, NDE1 and LIS1 in SC and *CEP85L* siRNA treated cells as expressed as the mean percentage ± s.d. of the fluorescence intensities of SC cells. **C.** Total cell lysates from U2-OS cells transfected with siRNA to SC or *CEP85L #1* or *#2* were immunoblotted for CEP85L, DYNC1H1, KIF2A, NDE1 and LIS1. Actin served as a loading control. **D.** U2-OS cells transfected with siRNA to *NDE1* or *KIF2A* co-stained with Centrin (green) and CEP85L (red). **E.** Mean fluorescence intensity of CEP85L in SC, *LIS1, NDE1,* and *KIF2A* siRNA treated cells expressed as the mean percentage of the fluorescence intensities of SC cells. **F.** U2-OS cells treated with DMSO or the Dynein inhibitor Ciliobreven co-stained for Centrin (green) and DYNC1H1 (red) or CEP85L (red). **G.** Quantification of the centrosomal fluorescence intensity of CEP85L in cells treated with DMSO or Ciliobreven expressed as the mean percentage of the fluorescence intensities of DMSO treated cells. **H.** U2-OS cells transfected with SC, *NDE1* #1-3, *KIF2A #1-3,* and *LIS1 #1-3* siRNAs blotted for CEP85L and NDE1, KIF2A or LIS1. α-tubulin served as a loading control. Boxed region represents specific bands. **I.** Western blot analysis of lysate from U2-OS treated with DMSO or Ciliobrevin probed with antibodies to DYNC1H1 and CEP85L. Actin served as a loading control. **J.** Immunofluorescence of SC and *CDK5* siRNA-transfected U2-OS cells co-stained for Centrin1 (green) and CDK5 (red) or pCDK5 (red). Scale bar represents 5μm for all images. **K.** Total cell lysate of U2-OS cells transfected with GFP or GFP-CEP85L for 5 hours were immunoblotted for GFP, CDK5 and pCDK5. Actin served as a loading control. **L.** Airyscan images of GFP and GFP-CEP85L (green) expressing U2-OS cells co-stained with antibodies to pCDK5 (red) and γ-tubulin (blue). Scale bar represents 1μm for Airyscan images. **M.** Quantification of the fluorescence intensities of CEP85L in WT and *CDK5 pat* cells expressed as the mean percentage of the fluorescence intensities of WT cells. **N.** Quantification of the mean fluorescence intensities ± s.d. of centrosomal Dync1h1, Kif2a, Nde1 and Lis1 in WT and *Cdk5*$^{-/-}$ cells as expressed as the mean percentage ± s.d. of the fluorescence intensities of WT cells. **O.** WT and *Cdk5*$^{-/-}$ mouse embryonic fibroblasts (MEFs) co-stained with antibodies to Centrin (green) and Dync1h1 (red), Nde1 (red), Kif2a (red), or Lis1 (red). **P.** Western blot analysis of whole cell lysates from WT and *Cdk5*$^{-/-}$ MEFs probed with antibodies to Cdk5, pCdk5, Dync1h1, Kif2a, Nde1 and Lis1. Actin served as a loading control. **Q.** Inverted images of WT and *Cdk5*$^{-/-}$ MEFs stained with α-tubulin, EB1 or acetylated tubulin. Triangles denote the location of the centrosome. Scale bar 5μm for all images. **R.**

Quantification of the fluorescence intensity of EB1 within 5μM of the centrosome expressed as the mean percentage ± s.d. of the fluorescence intensities of WT and *CDK5* patient fibroblasts. **S-T.** U2-OS cells treated with DMSO or Roscovitine were co-stained with antibodies to Centrin (green) and pCDK5 (red), DYNC1H1 (red), KIF2A (red), NDE1 (red), or LIS1 (red). **U.** Mean fluorescence intensity of pCDK5, DYNC1H1, KIF2A, NDE1, and LIS1 in DMSO or Roscovitine treated U2-OS cells expressed as the mean percentage of the fluorescence intensities of DMSO-treated control cells. For all quantifications, 10 cells were analyzed per experiment (n=3). * - $p < 0.005$ (Student's t-test). **V.** Western blot analysis of CDK5, pCDK5, DYNC1H1, KIF2A, NDE1, and LIS1 in cell lysates from DMSO and Roscovitine treated U2-OS cells. Boxed areas represent specific bands.